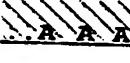


Fig. 1**O1**

LL44, LB68 853 860 870 880
 ...T G T T T A C T A A A A A T A T T T G G T A A A G C A
 ← ← → ← →
N62, LB10 ...T G T T T A C T A A A A G T A T T T G G T A A A A C A
 ← ← → ← →
N141  T G G C G A C T A A A A G T A T T T G G T A A A A C A
 ← ← → ← →
ISL7
N299  .A A A T T A C T A A A A A T A T T T A G T A A A A C A
 ← →
ISL5 ← →

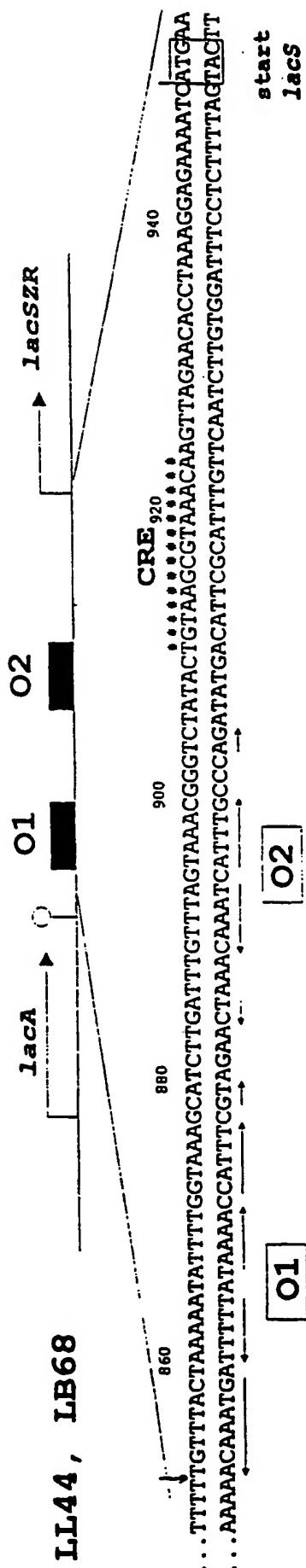
O2

LL44, LB68 890 900
 T C T T G A T T T G T T T A G T A A A C G G G T C T A T A...
 ← ← → ← →
N62, LB10 T C T T G A T T T G T T T A G T A A A C A A G T C T A T A...
 ← ← → ← →
N141 T C T T G A T T T G T T T A G T A A A C A A G T C T A T A...
 ← ← → ← →
N299 T C T T G G T T T A T T T A G T A A A C A A G T C T A T A...
 ← ← → ← →

Comparison of *L. delbrueckii* operators sequences (O1 and O2). Arrows are for inverted repeats. The LL44 sequence is numbered according to figure 1. Sequence of the second helix of *lacR* (repressor) is indicated.

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Fig. 2



3



Fig. 2 u. 3: Organisation of the promoter region of LL44 and N299 lac operon. Operators O1 and O2 are indicated by black boxes. The inverted repeats of the operators are represented by arrows.

The sequence responsible for catalytic repression (CRE) is overdrawn by stars.

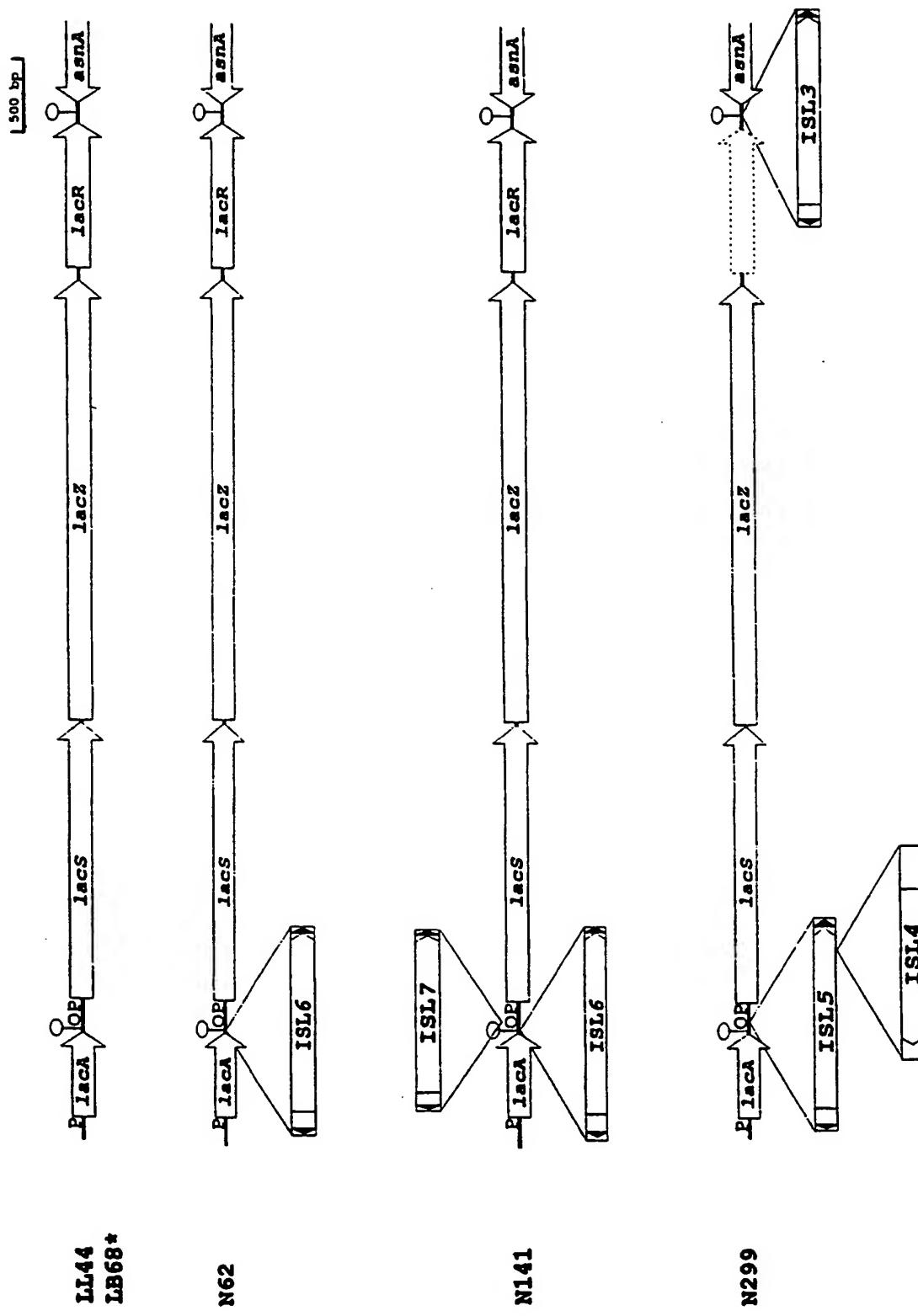
The sequence responsive for cathepsin D expression (CDS) is shown. The inverted repeat of ISL5 is boxed and shaded. The initiation of transcription is shown by an i (arrow head) (Leong-Morgenthaler et al., 1991). The promoter sequence of LL44 is numbered according to figure 1. The picture is not drawn to scale.

1 GAATTCTGCTGGATGCTCAGGAAGCCGCCAGCTCAAGCTGGTATTCAAGCCACTTT
 stop lacZ RBS
 61 ACTGATAATGCTACAATTGACTTAACAGCATAAATTTAGTAAAAGCGAGTGAAGAAG
 121 **ATGGCAACGATCAGAGAAGTGGCCAAGGCAGCCGGGTGCGCCAGCAGCGACGGTTCCGG**
 1 M A T I R E V A K A A G V S P A T V S R

 helix turn helix
 181 GTCTTGAACTATGACCAGACCCTGTCGGTCAATGAGGCAACGGCAGAAGATATTCAA
 21 V L N Y D Q T L S V N E A T R Q K I F K
 241 ACTGCTGAAGCCATGCACTACCATAAGAGCCGGAAAGACCAGAAAGAGCAAGC
 41 T A E A M H Y H K S R K T R K S K Q K R
 301 CTGGCGATCTGGCTGTGGTGTGACCAAGACCAGGGAGATCAAGGACCTCTATTACTATTCA
 61 L A I C L W C D Q D Q E I K D L Y Y Y S
 361 ATCAGAACCGCCGCAAGCAGAGGCAAGAAGCAGGGACTTGAAGGCCAGGTCA
 81 T T S A Q A E A K K Q G L E S Q V I Y
 421 CCGGCTGATCCTTGCCCGATCCAGCTGCTTAAGCGGGATTATCATGATTGGCTACCA
 101 P A D P L P D P A A L S G I M I G Y Q
 481 CAGTATTGCCAGACCGCTTGAATGAAGTCAAAAGTCTGGCTGCCCTGGTCTTGTC
 121 Q Y S P D R L N E V K K S G L P L V F V
 541 GATACTGACACCTAAATTGGGTTACTGCTCAGTGTGGCTGACTTGCCAGGCCATG
 141 D T D T L K L G Y C S V V A D F G Q A M
 601 CAGGAGGGCGTAGAGGTCTTGGGGCAGGGCAGGGAGCGGATGCCCTTTGGATGG
 161 Q E A L E V F W G Q G R E R I A L L D G
 661 GATTGGACAGTAATTGATAAAAACAATTGGTCACTCCGCTCCGATTATAAG
 181 D L D S N F D K N N L V D F R F R D Y K
 ▼
 721 AAGAGCCTCGCGCCCGCGGGCAGTACGACCCGGACTTAGTCTATGTTGAAACTTCACT
 201 K S L A A R G Q Y D P D L V Y V G N F T
 781 CCGCAATCTGGCTATGAAGCCATTAAAGAAGCTCTTAAGTCCGGCTCCTTCCC
 221 G A A G C C P Q S G Y E A I K E A L K S G S F P K A
 841 TTGATTGCCGCTAATGACGCCATTGGCTATTGGAGCATTGAAGGCCTTAAAGAAGCTGG
 241 L I A A N D A M A I G A L K A F K E A G
 901 ATTAAGTCCCAGGGACGTCACTGATTCTTTAAATGACACAACGGCAGCAGAATT
 261 I K V P E D V S L I S F N D T T A A E F
 961 GCCAACCCAGCCTTGACTAGCGTACATGTAGAGACCAGCAGATGGCCAGCCAGCGTC
 281 A N P A L T S V H V E T Q Q M G R A S V
 1021 AAGGTCATGAAAGACCTGCTGGATGATGATGAAGCCGGACTTACAAGGTCACTTCCCA
 301 K V M K D L L D D D E A G T Y K V T F P
 1081 ACAAAACTCGTTACCGGGAATCTTGGCCAAAAGCATAAGGCATAGAGCATAATAACAG
 321 T K L V Y R E S C P K A * →
 1141 CAAAGAAATAGCTTGGAGATTGATTTCTCCAAGCTATTTCTGTATATATTATGGCTGC
 stop asnA
 1201 ATTCTGTTGATCATTCTGGGAATGGGACAGCTTACGAACGTGGTCCAGCTTGCAGATC
 1261 CAGGCAATGACCCGTTCAAAG

Figure 4:

Nucleotide and amino acid sequences of the *L. delbrueckii* subsp. *lactis* LL44 *lacR* gene.
 Start (121) and stop (1119) codons are boxed. Putative *lacR* RBS is underlined.
 The putative rho-independent terminator is underlined by convergent arrows.
 Stop codons of the beta-galactosidase (*lacZ*) and Asn t-RNA synthetase (*asnA*) genes
 are boxed. Insertion sequence of ISL3 is represented by a large open arrow.
 Single base pair deletion (722) in the mutant LZL102 is shown by an arrow head,
 leading to a premature stop codon (758) underlined.

**Figure 5 :**

Physical map of the lactose operon of the different *L. delbrueckii* studied. Open arrows are for the *lac* operon genes and dashed arrow is for inactivated *lacR*. Boxes are for the different IS-elements, where the arrows heads are for the inverted repeats.
* same sequence as LL44 except an insertion in the 5' end of the *lacA* gene.

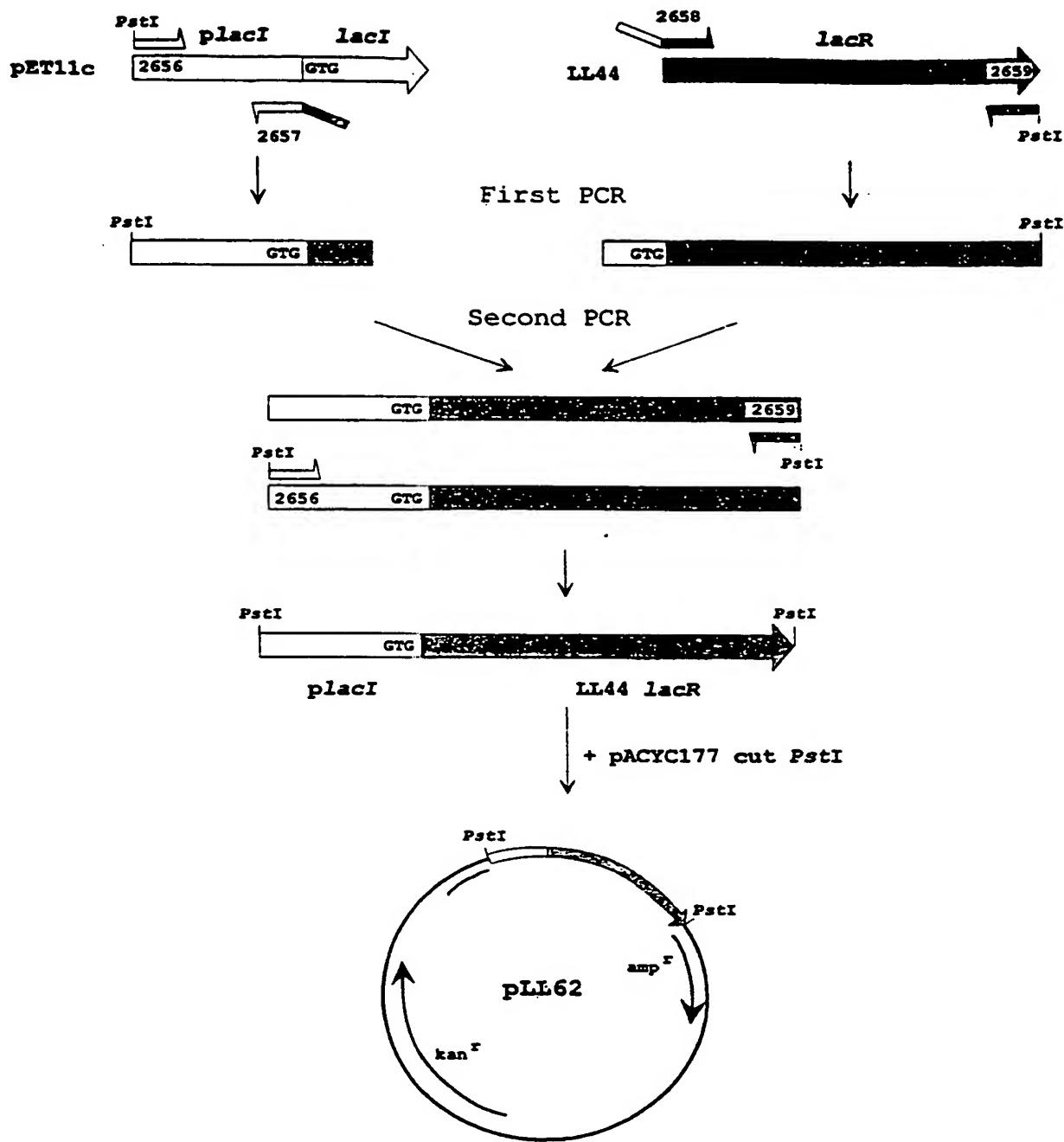


Figure 6 : Schematic representation of the construction of a pLL62. The dark box is for LL44 *lacR* gene and the white box is for the promoter region of the *lacI* gene of pET11c. Both were linked by PCR amplification using the SOEing method.

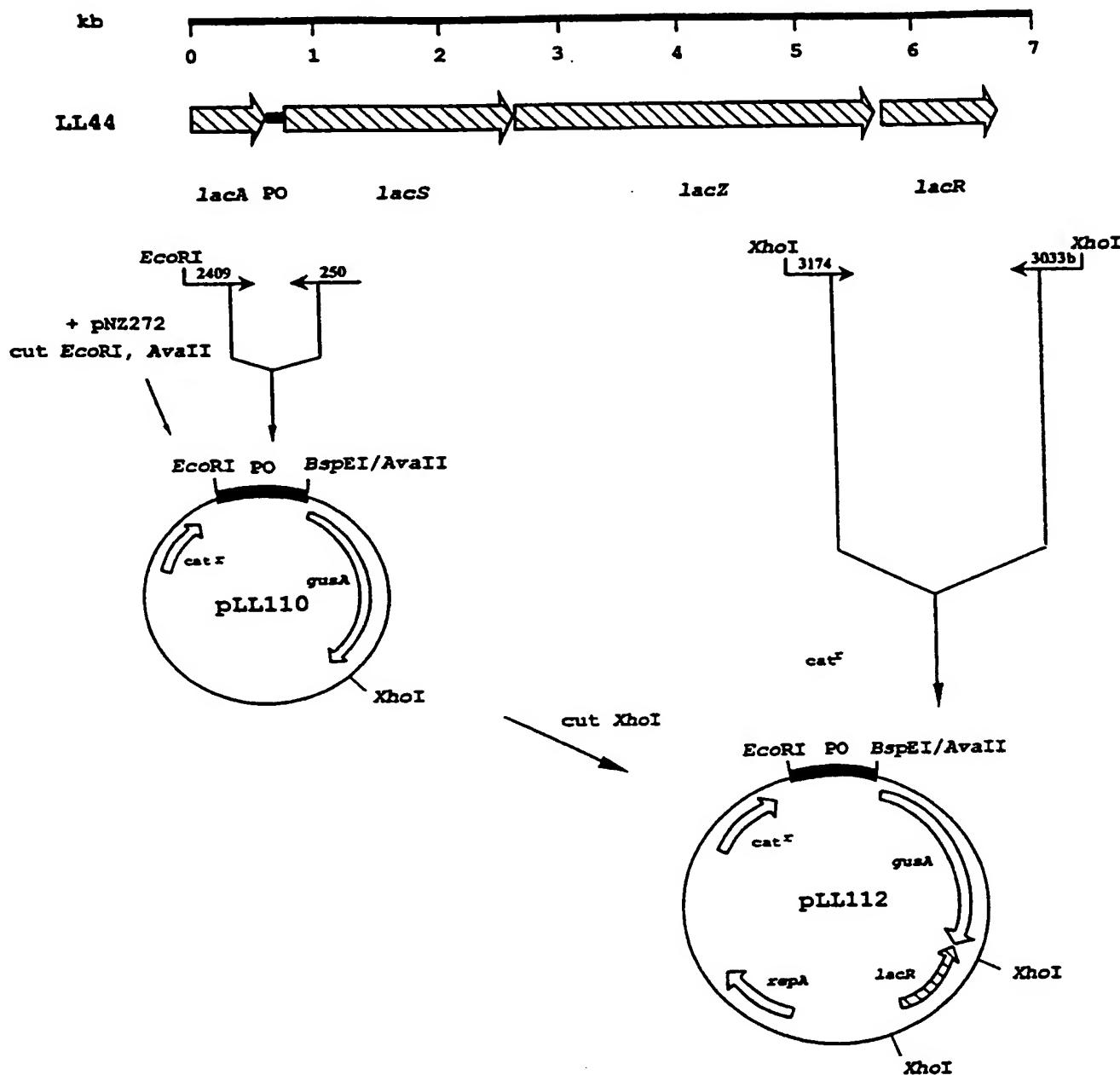


Figure 7 : Schematic representation of the construction of pLL110 and pLL112. Dashed arrows are for the genes of the *L. delbrueckii* lac operon, and open arrows for plasmid genes. The dark box is for the promoter region cloned in front of the *gusA* gene. Plasmids are not drawn to scale. The simple arrows represent the primers used to amplify the cloned regions.

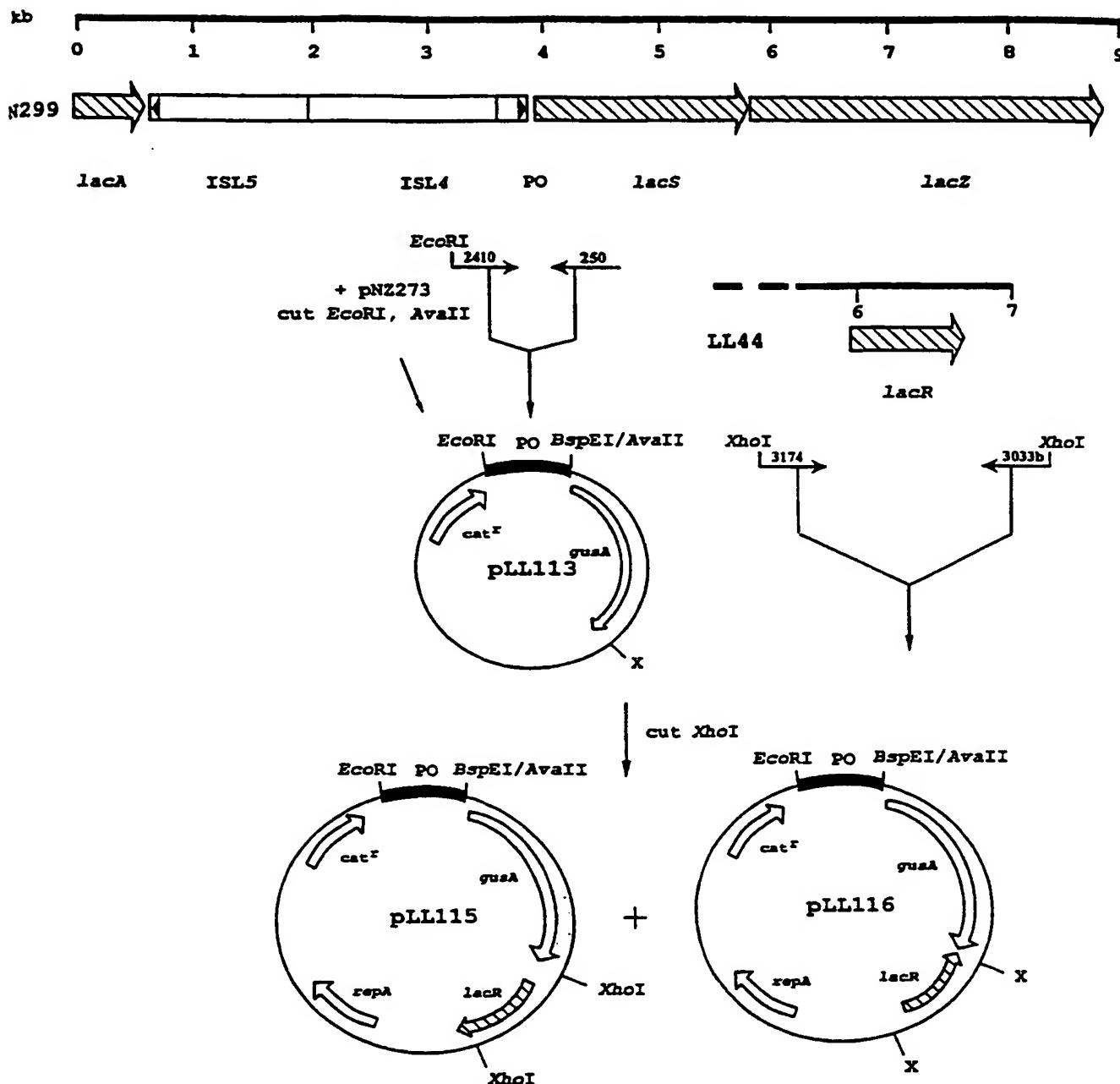


Figure 8 : Schematic representation of the construction of pLL113, pLL115 and pLL116. Dashed arrows are for the genes of the *L. delbrueckii* lac operon, and open arrows for plasmid genes. The open box containing arrow heads represents the IS-elements. The dark box is for the promoter region cloned in front of the gusA gene. Plasmids are not drawn to scale. The simple arrows represent the primers used to amplify the cloned regions.

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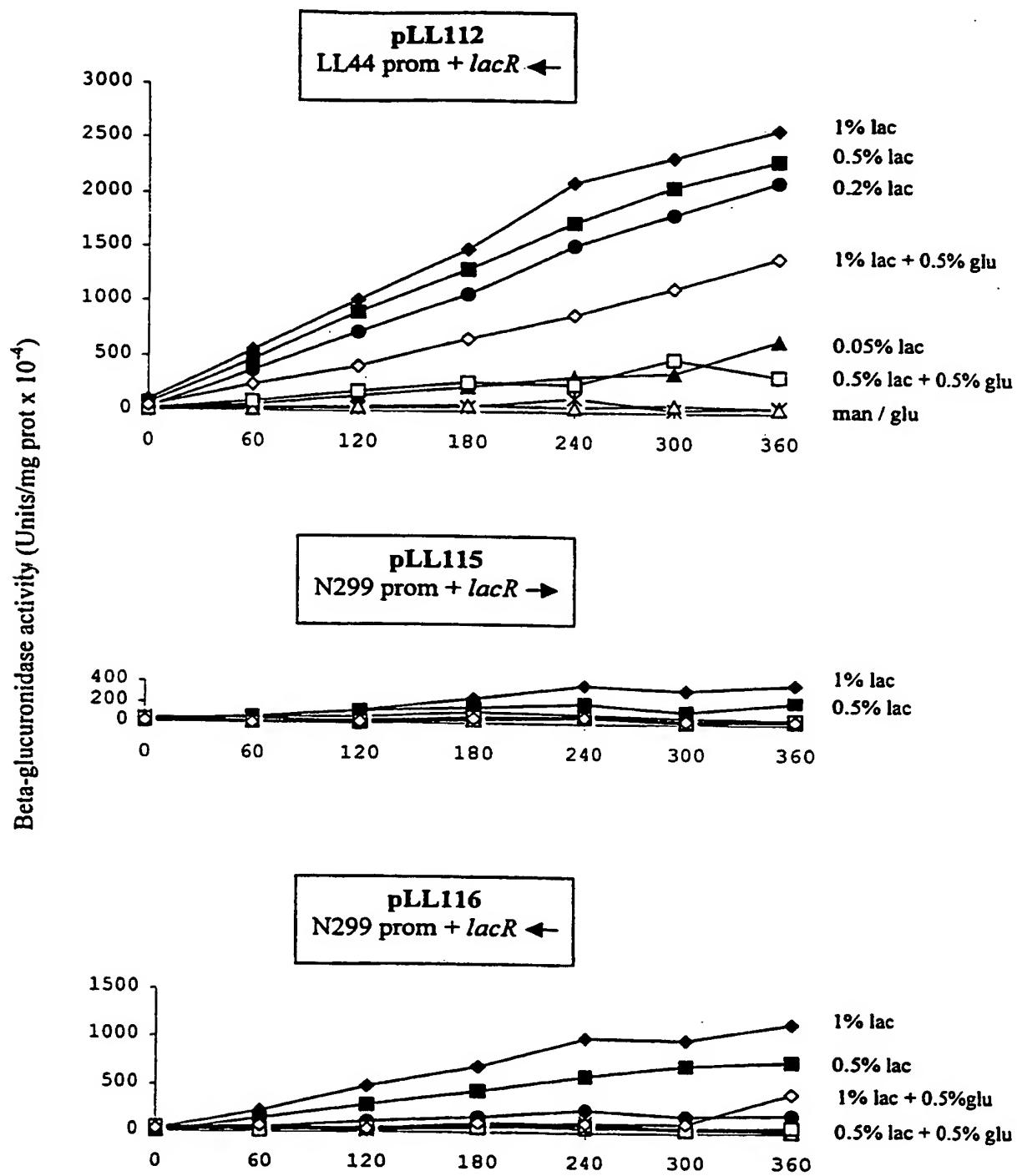


Figure 9 : Beta-glucuronidase activity (mean of three experiments) of *Lactococcus lactis* MG1363 containing different *Lactobacillus delbrueckii* lac promoter and the lacR gene of LL44. The lacR orientation compared to the gusA gene is represented by an arrow. The medium used was M17 containing : 0.5% mannose (×), 0.05% lactose (▲), 0.2% lactose (●), 0.5% lactose (■), 1.0% lactose (◆), 0.5% glucose (△), 0.5% glucose + 0.5% lactose (□) and 0.5% glucose + 1.0% lactose (◇).

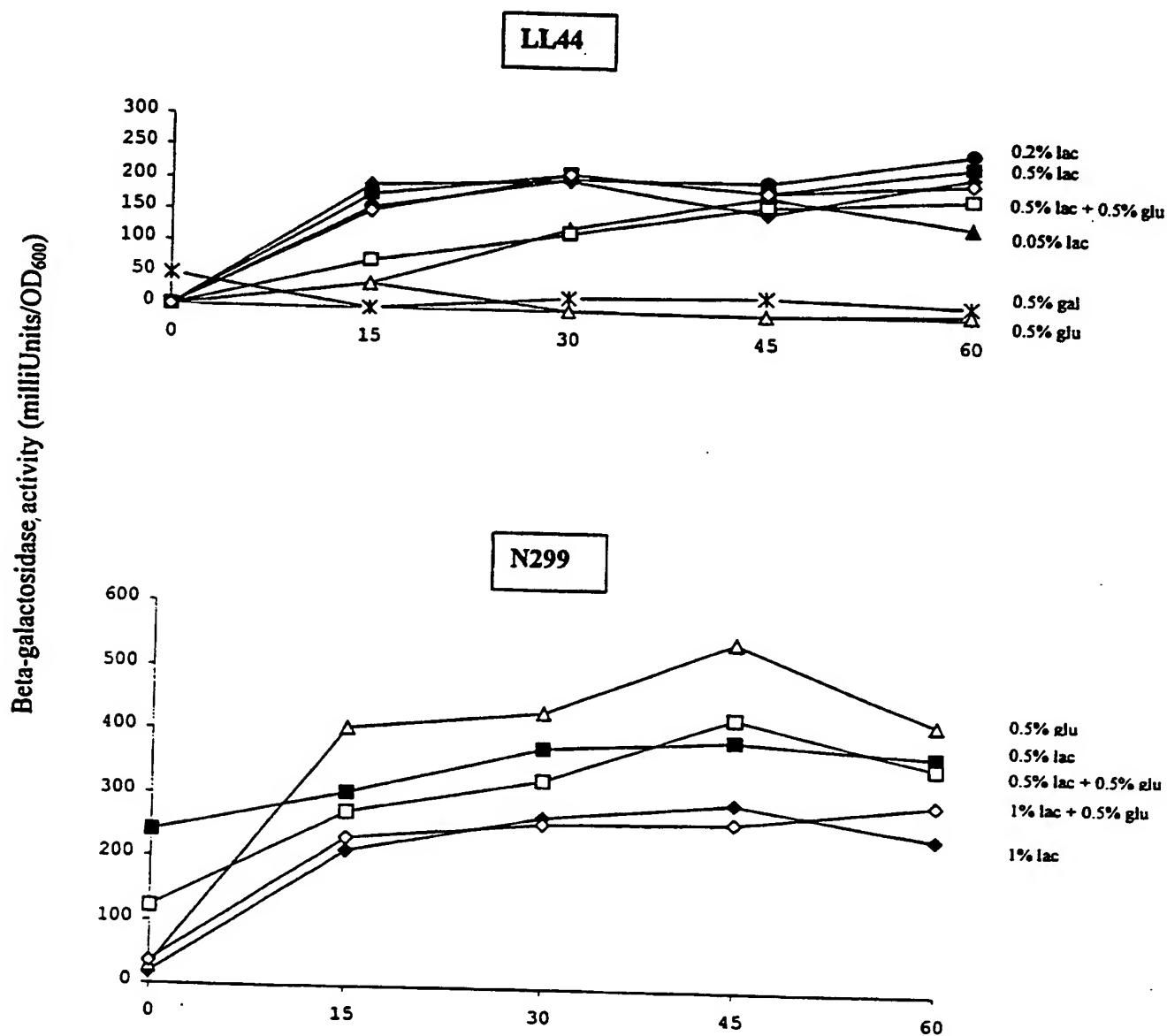


Figure 10: Beta-galactosidase activity (mean of three experiments) of *Lactobacillus delbrueckii* subsp. *lactis* LL44 and *L. delbrueckii* subsp. *bulgaricus* N299. The medium used was BHI-broth containing : 0.5% galactose (✖), 0.05% lactose (▲), 0.2% lactose (●), 0.5% lactose (■), 1.0% lactose (◇), 0.5% glucose (△), 0.5% glucose + 0.5% lactose (□), and 0.5% glucose + 1.0% lactose (◇). Strain N299 did not grow neither in galactose alone nor in 0.05% lactose and the experiment was not realised with these sugars.